METHODS FOR THE CULTURE, INFECTION, AND RECOVERY OF *CAPILLARIA OBSIGNATA* MADSEN, 1945

by

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INTRODUCTION

The demand for increased productivity and economy in agriculture necessitates development of new and better anthelmintics as well as the compilation of information concerning the actual damage inflicted by the various parasites and pathogens of domestic animals. In the case of worm parasites it is impossible to make studies which satisfactorily establish any of this knowledge without first having developed workable techniques for the maintenance and standardization of infections in the normal host. Such techniques have been developed to approach perfection in many instances, but workable techniques are not known for some of the common parasites of domestic animals.

Capillaria obsignata Madsen, 1945, has been known for a number of years as a parasite of chickens; however, there are few publications reporting experimental research with this worm and only one dealing with techniques which appear workable in the laboratory.

The objective of this study was to elucidate further the problems which might be encountered in the use of previously described techniques and to devise means for overcoming such problems.

REVIEW OF LITERATURE

Graybill (1924) was the first to observe C. obsignata in the United States, and he reported the successful embryonation of eggs from gravid females cut into pieces and placed in a shallow layer of physiological saline. Vermiform embryos were developed in six to seven days when cultured at room temperatures (22-25° C.). He did not infect chickens with these eggs.

Cram (1932) reported the embryonation of eggs obtained from gravid female C. obsignata and incubated for 40 days. When these eggs were fed to a quail and a chicken, eggs of the worm were found in the host feces in 21 days.

In 1936 Levine reported the use of fly larvae to digest the putrescible material from the feces of chickens and pigeons infected with C. obsignata. The resulting residue containing Capillaria eggs was then cultured. Subsequently, chickens were infected with these eggs by mixing the residue with their feed. Levine (1938) also reported recovery of Capillaria from chickens by scraping the intestinal mucosa into a dish and examining the material microscopically. He reported that in moderate infections the worms were only found in the lower two thirds of the small intestine, but in heavy infections worms could be found in all portions of the intestine including the duodenum and caeca. Infections with 1500-5000 worms were noted in a few fatal cases, but none of the chickens in which nonfatal infections were established harbored more than 14 worms.

Rowan and Gram (1959) reported the successful separation of

helminth eggs from relatively large samples of feces and sewage through the use of an apparatus consisting of a settling tray over which the feces and water mixture was allowed to flow.

Marquardt (1961) utilized gradient centrifugation to obtain nematode eggs from a concentrate derived by sieving and centrifuging a feces-water mixture.

Hill and Zimmerman (1961) used a specially constructed device to separate nematode eggs from feces and considered it to be a faster and more convenient method than that of Rowan and Gram.

Frazier (1962) reported the successful embryonation of eggs obtained from adult C. obsignata by cutting the worms into small pieces and incubating in 1% formalin for 6 days at room temperature. The culture was continually agitated by a mechanical shaker. Eggs were also obtained from the feces of infected chickens by a technique utilizing a combination of flotation in heavy liquids, passage through fine sieves, sedimentation by dilution of the solution, and centrifugation as needed. It was found that 95% of the eggs obtained from fresh feces would embryonate when incubated at room temperature using a water bath shaker. When these eggs (200) were inoculated into the crops of chickens, more than 90% developed to adults. Age of the host seemed to be the most influential single factor conditioning infections, with 3-8 weeks being optimum. Adult Capillaria were recovered from the intestine after 19 days. His technique for recovery of worms consisted of making a longitudinal slit in the excised intestine, soaking it in 5% saline solution for one

hour, then washing it with a fine spray of water under high pressure.

METHODS AND MATERIALS

Capillaria obsignata Stock Material

Several hundred mature female *C. obsignata*, and also an egg culture containing several thousand larvated eggs were obtained from Dr. Salsbury's Laboratories, Charles City, Iowa. The eggs had been separated from feces of infected chickens, and were contaminated with eggs of *Ascaridia galli* Schrank,1758, and *Heterakis gallinarum* Schrank, 1758, as well as oocysts of *Eimeria tenella*.

Chickens

In all experiments, except number 4, straight-run White Leghorns were obtained from either Manhattan Hatchery or DeForrest Hatchery. In Experiment 4, White Rock chickens were used. On receipt at the laboratory, the chicks were given intranasal Newcastle vaccine, and maintained in electrically heated brooder batteries for two weeks before they were transferred to larger cages. All birds were fed a standard antibiotic free commercial ration. Chickens were infected when they were two to five weeks old.

Egg Culture

Three basic methods, with some additional variations, were

used for the preparation of egg cultures. In one method various means were employed to reduce whole worms to small pieces before incubating. One culture was prepared by cutting female worms into small pieces in a dish of 1% formalin. The resulting material was then transferred to a small flask. Aluminum foil was used to exclude light. The culture was incubated for one week at room temperature (26-29°C.), while being continuously agitated on a gyrotory shaker. This culture was used in Experiment 1. In other instances, a TenBroeck tissue grinder or a VirTis Model 23 homogenizer was used to break worms into small pieces. When using the TenBroeck apparatus, the worms were ground until visual observations indicated that only very small pieces remained. The cultures were incubated for two weeks. Three egg cultures were prepared in this manner and were used in Experiments 2 and 3. In using the VirTis homogenizer, the worms were placed in the special 30 ml. homogenizing flask in about 25 ml. of 1% formalin and homogenized for five minutes with the rheostat control set between "medium" and "high." The resulting material was incubated in a petri dish at 32°C. for two weeks. No mechanical agitation was employed. Egg cultures prepared in this manner were used in Experiment 8.

The second basic method for the preparation of egg cultures consisted of incubating whole mature female worms at 32°C. in a 1% formalin solution. Two weeks were allowed for development, after which the cultures were stored at 7°C. Nine cultures were prepared in this manner, and were used in Experiments 4, 5, and 6.

The third method attempted involved the concentration of eggs in the feces of infected chickens. Feces were collected and stored in jars at 7°C. Before concentration procedures, a small amount of the feces was examined by direct flotation to estimate the egg content. The first extraction procedure tested consisted of the following steps. Feces were mixed 1:4 by volume with water in a Waring blender for two minutes. The mixture was strained through a series of eight sieves (10, 20, 30, 40, 60, 80, 100, 150, and 200 mesh). The filtrate was poured into Pilsner glasses and allowed to settle for 30 minutes before the supernatant was siphoned off and clean water was added. This procedure was repeated twice. The sediments were combined and enough sugar was added to produce a 1.30 molar solution. The material was centrifuged at 1000 rpm for five minutes. The supernatant solutions were combined, diluted 4:1 by volume with water, and poured into Pilsner glasses. After 30 minutes the sediments were collected and examined for the presence of eggs of C. obsignata.

The second procedure tested was conducted in the following manner. Feces were blended with water in a Waring blender in a 1:1 ratio. The mixture was washed through a series of seven sieves (10, 20, 40, 80, 100, 150, and 200 mesh), with a spray of water under high pressure. The filtrate was allowed to stand in large containers for several hours or overnight, then the supernatant fluid was siphoned off and discarded. The sediment was concentrated to about 400 ml., and diluted to 1,000 ml. with saturated sodium nitrate solution (sp. gr. ca. 1.34). After

having been thoroughly mixed, the solution was centrifuged at 1000 rpm for ten minutes. The supernatant was transferred to Pilsner glasses and diluted 4:1 by volume with water. After 30 minutes the sediment was collected and examined microscopically for the presence of eggs.

The third procedure used for the concentration of eggs in feces is as follows. The feces were blended 1:1 with tap water, then washed through 40, 80, and 150 mesh sieves by a high pressure spray of water. The resulting solution was allowed to stand in large containers for at least 24 hours. The sediment was collected and concentrated by centrifuging at 1000 rpm for five minutes. This was diluted 5:1 with saturated sodium nitrate solution, then centrifuged at 1500 rpm for 30 minutes. The supernatant was collected, diluted 6:1 with tap water and allowed to stand for 24-48 hours before the sediment was collected and examined microscopically for the presence of eggs. Samples were taken after each step in the procedure and examined in an attempt to follow the fate of the eggs.

In a variation of the above procedure, feces were blended 1:1 by volume with water. The mixture was washed through a series of three sieves (40, 80, and 150 mesh) with water under high pressure. The resulting solution was centrifuged at 2000 rpm for 15 minutes. A portion of the supernatant was retained for examination, and the sediment was collected for further processing. The sediment was blended with saturated sodium nitrate solution in a ratio of one part sediment to five parts sodium

nitrate. The mixture was centrifuged at 2000 rpm for 30 minutes, after which the supernatant was collected. A portion of the sediment was retained for examination. The supernatant was diluted 5:1 with water and centrifuged at 2000 rpm for 15 minutes. A portion of the supernatant was retained as a check, and the sediment was collected and examined for the presence of eggs. To assess the egg content of the concentrate 0.2 ml. portions were examined in the manner described by Frazier (1962). The sample was placed in a 12 ml. centrifuge tube. The tube was then filled to the rim with saturated sodium nitrate solution and a coverslip placed on the rim. This was centrifuged at 1500 rpm for 10 minutes. All the eggs adhering to the coverslip were counted. This procedure was repeated twice and an average egg count was calculated.

Infection Techniques

The nature of different types of egg cultures necessitated the use of different infection techniques. The techniques were conditioned by the use of free eggs or the use of eggs contained in whole worms.

Calibration of egg dose when using free eggs was accomplished by counting the eggs in 0.2 ml. of stock solution delivered from the same pipette used for infection. The final egg count was an average of three such counts. Infection was accomplished by inserting the pipette into the crop of the chicken and discharging the contents of the pipette as it was removed.

The use of sucrose solution as a stock solution medium was

investigated. It was recognized from the work of Hansen, et al (1954) that egg doses could be estimated more accurately when a sucrose solution of the same density as the eggs was used as a suspension medium. In the present study, by observing the direction of movement of the eggs placed in sucrose solutions of different concentrations (1.25, 1.26, 1.27, and 1.28 M.) the density of the eggs was determined to be equal to that of a 1.27 M. sucrose solution. In practice, a water suspension of eggs, which had been concentrated to twice the desired dosage, was mixed 1:1 with a 2.5 M. sucrose solution.

When eggs cultured in whole worms were used, a different technique was necessary. The desired dosage size was first approximated by counting the embryonated eggs present in the uterus of each female worm with the aid of a compound microscope (100X). Then each dose was accurately counted into a small amount of water in black porcelain spot plates. An eyedropper pipette was used to administer the doses to chickens. Records were kept of the exact number of eggs given to each chicken.

Recovery of Worms

Chickens were necropsied at 25-61 days post-infection. The portion of the intestine between the gizzard and the yolk sac diverticulum was removed and slit lengthwise with scissors and the intestinal contents were rinsed into a dish of tap water. In Experiment 3, the dish of water containing the intestinal debris was retained and examined to determine if any worms were lost by this treatment. The intestine was then

placed in a dish of 5% saline solution for one hour, after which it was poured into a 150 mesh sieve. A high pressure spray of water was used to wash the intestine for one to two minutes. The intestine was discarded and the contents of the sieve were washed into a jar. Sufficient formalin was added to each jar to make a 1½% solution. The jars were stored in the refrigerator at 7°C. until the worms were removed and counted. In Experiment 9, the intestines were retained and examined microscopically for worms which may not have been recovered by the treatment.

A second method tested differed somewhat from the above method. The intestine was removed, slit longitudinally, and rinsed to remove debris. Sections were placed in a 1% solution of hydrochloric acid (by volume) at 40°C. Stirring was provided by a magnetic stirrer until all of the mucosa had been disrupted. The solution was then allowed to stand in a large Baerman type funnel for 30 minutes. The sediment was drawn off by releasing a stopcock on the spout of the funnel. The sediment was examined for the presence of worms. Only one necropsy was performed in this manner.

Counting and Storage of Worms

A widefield binocular dissecting microscope with 7X magnification was used for the examination of the above mentioned material. Small portions of the material were poured into specially prepared petri dishes, the bottoms of which were marked with parallel lines spaced about one cm. apart. A black background was used and lighting was provided by a fluorescent lamp

placed to one side. Worms were removed from the petri dish on the tip of a dissecting needle, counted, and placed in a small dish of water. The worms were stored in 1% aqueous formalin at 7°C. until used for preparation of egg cultures.

RESULTS

Egg Culture

Incubation of female worms yielded an average of 28.24 embryonated eggs per worm. Incubation of chopped worms yielded an average of 3.75 embryonated eggs per worm, whereas, cultures prepared by grinding worms in a TenBroeck tissue grinder yielded 7.05, 7.14, and 14.70 embryonated eggs per worm, respectively, for the three cultures.

The use of the VirTis homogenizer in preparing egg cultures was evaluated in the results of two such preparations. One culture yielded an average of 8.86 embryonated eggs per worm, whereas, the other culture yielded 21.36 embryonated eggs per worm.

Fecal concentrates obtained by the procedure described were found to contain fewer eggs per unit volume than the original fecal material. Egg cultures prepared in this manner were not used to infect chickens.

Experimental Infection of Chickens

Experiment 1

Twenty 21-day-old birds were used in this experiment. One

group of three birds received about 100 embryonated eggs each. These eggs were from a culture of small pieces of worms. The other group of 17 birds received 180 embryonated eggs each from a culture supplied by Dr. Salsbury's Laboratories. A 1.27 M. sucrose solution was used to suspend eggs from both types of cultures during inoculations.

Necropsy was performed at 31 to 61 days post-exposure. Recovery of worms was accomplished by the method using a high pressure spray of water, except for bird #731, from which worms were recovered using the settling funnel technique.

The worm burden of chickens in group one averaged 52.33, or 52.33% of the eggs administered. Chickens in group two harbored an average of 39.76 worms per bird or 22.09% of the embryonated eggs administered (Table 1).

It was interesting to note the relationship between the number of days post-exposure before necropsy and the observed worm burden. The worm burdens at necropsy were larger in the birds which were examined 31 to 40 days post-exposure than in birds examined 45 to 61 days post-exposure (Fig. 1).

Experiment 2

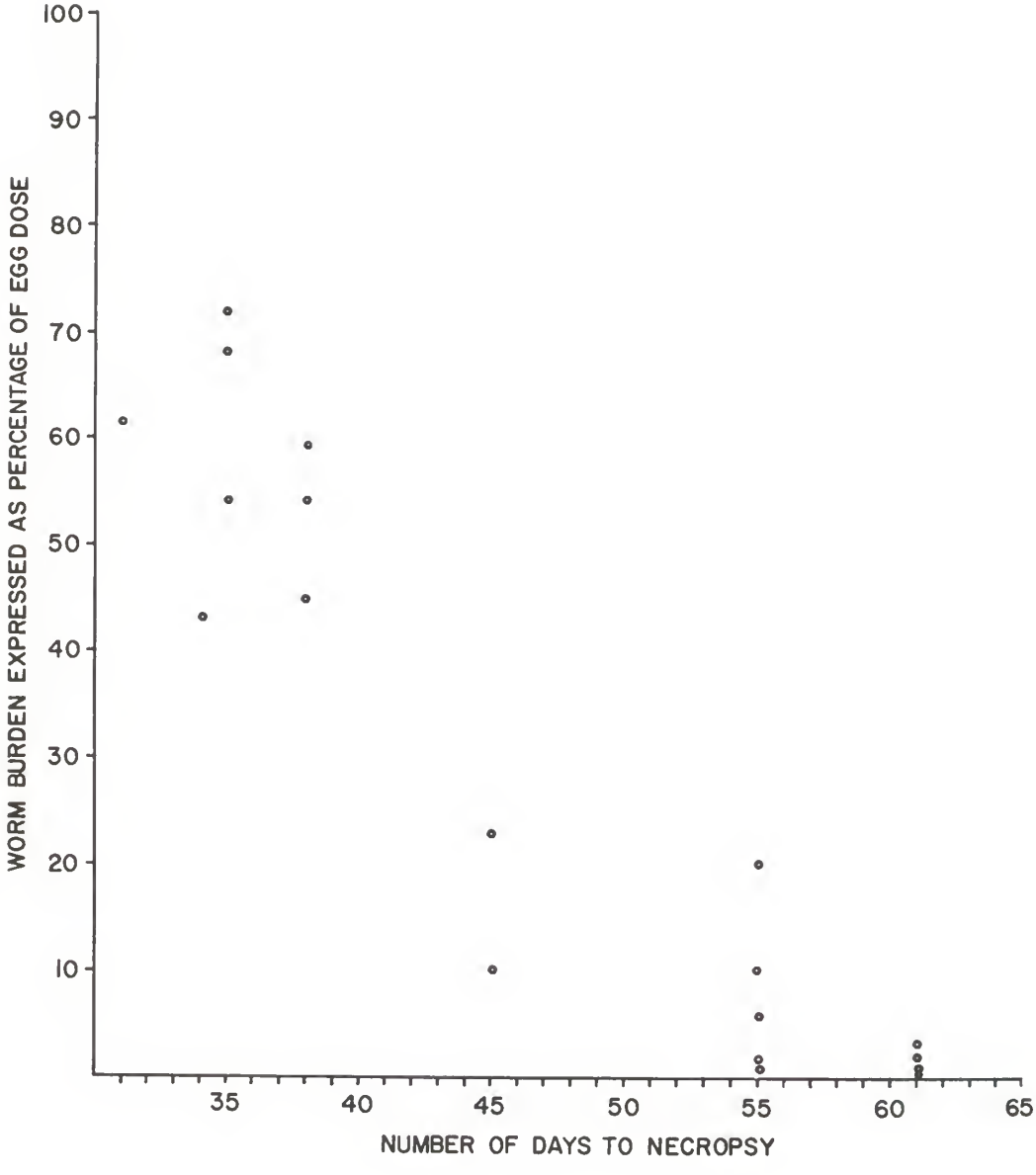
Seven 5-week-old birds were infected with 200 embryonated eggs of C. obsignata. The egg cultures used were those prepared with the TenBroeck tissue grinder and dispersed in distilled water during the infection procedure.

Necropsies were performed at 25 days post-exposure in the same manner as in Experiment 1. The average worm burden was

Table 1. Infectivity of Capillaria obsignata when chickens were given 100 or 180 embryonated eggs.

Group	Chicken Number	Egg Dose	Days to Necropsy	Worms Recovered
I	723	100	38	59
	740	"	38	55
	741	"	34	43
	Total:			157
	Average:			52.33
II	724	180	61	1
	725	"	56	10
	726	"	35	97
	727	"	61	8
	728	"	56	1
	729	"	45	18
	730	"	38	80
	731	"	45	43
	732	"	56	18
	733	"	35	128
	734	"	61	2
	735	"	31	110
	736	"	61	1
	737	"	35	122
	738	"	56	32
	739	"	56	2
	742	"	61	6
	Total:			679
	Average:			39.76

Figure 1. Observed worm burdens from Experiment 1, expressed as percent infectivity, plotted against the number of days to necropsy.


WORM BURDEN EXPRESSED AS PERCENTAGE OF EGG DOSE
100
90
80
70
60
50
40
30
20
10
35
40
45
50
55
60
65
NUMBER OF DAYS TO NECROPSY

20.71 (10.36%)(Table 2).

Experiment 3

Five 5-week-old birds were infected with 200 embryonated eggs in the same manner as in Experiment 2. Necropsies were performed 31 days post-exposure. The worm burden averaged 59.60 per bird or 29.80% of the embryonated eggs administered (Table 3).

Experiment 4

Five birds were infected with approximately 200 embryonated eggs each from cultures of whole worms (Table 4). These birds were 16 days old at the time of infection and they received an immunosuppressant (hydrocortisone) given intramuscularly at intervals of 3 to 5 days during the course of the infection. The dosage was 0.625 mg./dose in 0.1 ml. of buffered saline.

Necropsies were conducted 30 days post-exposure according to the procedures used in previous experiments. An average of 2 worms per bird (0.95%) was recovered at necropsy.

Experiment 5

A total of 24 birds were divided into three groups of eight birds each. Birds of group I received about 200 embryonated eggs from whole worm cultures taken from the refrigerator and given directly without warming. The exact number of eggs in each dose is shown in Table 5. Birds of group II each received about 200 eggs using the same procedure as in group I except that the

Table 2. Chickens inoculated with 200 embryonated eggs of Capillaria obsignata.

Chicken Number	Egg Dose	Days to Necropsy	Worms Recovered
1155	200	25	20
1156	"	"	56
1157	"	"	5
1158	"	"	11
1159	"	"	18
1160	"	"	33
1161	"	"	2
	Total:		145
	Average:		20.71

Table 3. Chickens inoculated with 200 embryonated eggs of Capillaria obsignata.

Chicken Number	Egg Dose	Days to Necropsy	Worms Recovered
1301	200	31	25
1302	"	"	89
1303	"	"	62
1304	"	"	38
1305	"	"	84
	Total:		298
	Average:		59.60

Table 4. Chickens infected with eggs from whole worm cultures and treated with hydrocortisone

Chicken Number	Egg Dose	Days to Necropsy	Worms Recovered
1505	224	30	6
1506	234	"	1
1507	193	"	0
1508	229	"	1
1509	170	"	2
	Total:		10
	Average:		2

Table 5. Birds infected with 200 or 400 eggs from cultures prepared with whole worms

Group	Chicken Number	Egg Dose	Days to Necropsy	Worms Recovered
I	1510	195	34	0
	1511	206	"	4
	1512	191	"	1
	1513	199	"	1
	1514	194	"	2
	1515	174	"	3
	1516	199	"	0
	1517	188	"	2
	Total:			12
	Average:			1.5
II	1518	191	34	0
	1519	215	"	2
	1520	204	"	0
	1521	189	"	0
	1522	209	"	0
	1523	212	"	0
	1524	207	"	0
	1525	170	"	8
	Total:			10
	Average:			1.25
III	1526	415	34	0
	1527	389	"	0
	1528	398	"	1
	1529	408	"	0
	1530	390	"	2
	1531	416	"	0
	1532	409	"	0
	1533	382	"	0
	Total:			3
	Average:			0.38

the egg culture was warmed in an incubator at 31 C. for two hours prior to use. Birds of group III each received about 400 eggs according to the procedure used for group II.

Necropsies were performed after 34 days. In group I, the average worm burden was 1.62 worms per bird (0.81%). Chickens in group II had worm burdens averaging 1.25 (0.63%). Birds of group III harbored an average of 0.38 (0.19%) worms per bird.

Experiment 6

Ten birds, 3 weeks old, were divided into two groups of five birds each. The birds in group I each received about 100 eggs from a culture prepared with whole worms (Table 6). Each bird in group II received 100 eggs from the egg culture supplied by Dr. Salsbury's Laboratories.

Necropsies performed 34 days post-inoculation revealed that each bird of group I harbored an average of 11.8 (10.84%) worms. Each bird of group II harbored an average of 83 worms (83%).

Experiment 7

Nine chickens were divided into three groups of three birds each. At six weeks of age, the birds of group one each received 90 embryonated eggs from the egg culture acquired from Dr. Salsbury's Laboratories. Birds in group two each received 180 eggs from the same culture, and birds of group three each received 270 eggs from the same culture. The eggs were inoculated into chickens from a stock solution using distilled water as the dispersing medium.

Table 6. Chickens inoculated with eggs from cultures prepared from whole worms (group I) and cultures prepared from free eggs (group II)

Group	Chicken Number	Egg Dose	Days to Necropsy	Worms Recovered
I	1901	102	34	4
	1902	115	"	3
	1903	83	"	24
	1904	129	"	21
	1905	115	"	7
	Total:			59
	Average:			11.8
II	1906	100	34	71
	1907	"	"	70
	1908	"	"	97
	1909	"	"	80
	1910	"	"	97
	Total:			415
	Average:			83

Necropsies were performed at 21 days post-infection in the same manner as in previous experiments (Table 7). The observed worm burdens of the birds of group I averaged 39 (43.33%) worms per bird. The birds in group II harbored an average of 110.66 (61.77%). The observed worm burden for birds in group III averaged 134.33 (49.63%).

Experiment 8

Nine chickens were divided into groups of three birds each. At 25 days of age the birds were given embryonated eggs of C. obsignata. The birds of group I each received 100 eggs, birds of group II each received 200 eggs, and birds of group III each received 300 eggs (Table 8). The egg culture used was prepared with the VirTis Homogenizer; the eggs were suspended in distilled water for inoculation.

Necropsies were performed 40 days post-inoculation in a manner previously described. The worm burdens detected in birds of group I averaged 30 (30%). The worm burdens of chickens in group II averaged 22.66 (11.33%). Birds of group III yielded an average of 30 (10%) worms each.

Table 7. Chickens inoculated with 90, 180, or 270 embryonated eggs, respectively.

Group	Chicken Number	Egg Dose	Days to Necropsy	Worms Recovered
I	1938	90	25	57
	1939	90	25	26
	1943	90	25	34
	Total:			117
	Average:			39
II	1937	180	25	141
	1941	180	25	122
	1942	180	25	69
	Total:			332
	Average:			110.66
III	1944	270	25	101
	1945	270	25	223
	1946	270	25	79
	Total:			403
	Average:			134.33

Table 8. Chickens inoculated with 100, 200, or 300 embryonated eggs, respectively.

Group	Chicken Number	Egg Dose	Days to Necropsy	Worms Recovered
I	2240	100	40	26
	2233	100	40	32
	2226	100	40	32
	Total:			90
	Average:			30
II	2238	200	40	22
	2239	200	40	32
	2227	200	40	14
	Total:			68
	Average:			22.66
III	2231	300	40	7
	2237	300	40	57
	2239	300	40	26
	Total:			90
	Average:			30

Discussion

In many industrial and institutional parasitological laboratories, the use of Capillaria obsignata as a test parasite has been handicapped because of difficulty in propagation. The difficulties have been associated with the small number of eggs in the uteri of females and the inability to effectively concentrate eggs from the feces.

The highest average yield of embryonated eggs was obtained from cultures prepared from whole worms. It was noted that upon incubation, all of the eggs in the anterior portions of the uteri would develop coiled embryos. Eggs posterior to a ceratin point (a different point in each worm) would fail to undergo cleavage, even though they appeared to be normal. Possibly the eggs that did not undergo cleavage were not fertilized. Separation and culture of the eggs under different conditions (aeration, etc.) might indicate whether fertilization or some other factor is involved. If it is assumed that all of the fertilized eggs developed then such information is of value in evaluating the yield of other culture methods.

Egg cultures prepared from whole worms, although yielding the highest number of embryonated eggs for each worm, were of lower infectivity when administered to chickens (Table 6). The egg culture prepared from free eggs showed a rate of infectivity seven times as great as the whole worm culture. It seem probable that the cuticle of the worm had some effect in delaying the stimulus which initiates hatching. In addition, the infective

larvae would have had some difficulty in escaping the confines of the parent worm before peristalsis carried them through the small intestine. This matter might be further elucidated by the use of a drug such as atropine sulfate to retard peristaltic action thereby allowing the eggs more time in the small intestine. When the VirTis homogenizer was used for preparation of egg cultures, as high as 21.36 embryonated eggs per worm were obtained. Subsequent infection of chickens produced worm burdens which were much lower than those normally observed when using eggs from feces.

Preparations made using the TenBroeck apparatus yielded an average of 9.34 eggs for each female worm. Subsequent infection of chickens showed a low degree of infectivity. The grinding process is suggested as a highly damaging procedure; microscopical examination after treatment revealed many egg fragments.

Observations during this work did not support the statement of Frazier (1962) that constant agitation is absolutely necessary for the successful embryonation of eggs during incubation. It is possible that his statement was made in reference to the incubation of concentrates of fecal material. The decomposition activities of bacteria could seriously lower the availability of oxygen to the developing embryos at a critical time. Such action can be controlled in cultures which do not contain large amounts of fecal material (e. g. whole worm cultures, etc.).

In the present study, procedures for the concentration of eggs in feces were not sufficiently developed to be productive. During tests of the various techniques used, samples were taken

after each step so that an evaluation of the procedure could be made. It was found that few eggs were lost in washing the material through sieves. Each subsequent step, however, allowed loss of eggs, resulting in a fecal concentrate containing only a few eggs. The inability to reproduce the results reported by Frazier (1962), Rowan and Gram (1959), and other workers is attributed partly to the inavailability of equipment similar to that used by them. Frazier reported the use of speeds up to 11,000 rpm in centrifuging, whereas in the present study the maximum speed attained was 2,000 rpm. It is possible that refinement of centrifuge techniques could give better results. It was noted that when the centrifuge was allowed to stop without gradual deceleration there was much swirling of the liquid in the tubes. Some difficulty was realized in obtaining feces with high egg content. In the present study the feces used was collected from chickens with worm burdens of 20-100. Possibly the feces from "heavily infected birds" used by Frazier were from chickens with a much greater worm burden.

Calibration of egg dosage and infection procedures were conveniently accomplished by the techniques described when using either chopped worms or eggs from feces. Frequently, however, it was noted that small pieces of worms in the cultures contained several eggs. This is similar to the "clumping" problem experienced in work with other parasites when using water suspensions (Hansen, et al, 1954). It was not desirable to attempt a complete separation of the eggs because the harsh treatment needed would destroy large numbers of them. Such unavoidable

clumping would detract from the advantage of using 1.27 M. sucrose solution as the inoculation medium to improve statistical accuracy, since one of the advantages of using such a preparation with other kinds of worms is the reduction of the tendency to clump.

Calibration of egg dosage when using whole worms was very time consuming. If large variations in the size of egg dosage can be tolerated, the average number of eggs in each female worm can be used to determine the number of worms necessary for each dose. By counting the eggs in each worm for each egg dose a highly accurate determination of dose size can be made. Since the number of embryonated eggs contained by the worms varied from 0 to 75, however, it was sometimes difficult to select worms in which the total number of eggs was within reasonable closeness to the desired dosage.

The recovery of worms from infected chickens, when using the method of Frazier (1962), is easily done. Subsequent examination of intestines did not reveal any worms that had been left attached to the mucosa.

In some experiments the intestines were not rinsed free of debris before removal of worms. In these instances the debris, which included particles of feathers bearing strong resemblance to the worms, seriously hampered collection of worms. In one experiment the rinse water was retained and examined for worms that had been lost. No worms were found.

The Baerman funnel apparatus was used in only one instance with unsatisfactory results. When either of the techniques described was used, much of the mucosa was removed in the treatment.

Upon examination, the material was found to contain many entangled worms. Such material ordinarily floated. This would result in the loss of the worms if only the lower alliquot were taken from a Baerman funnel apparatus.

Johnson (1962) reported that the use of an immunosuppressant such as hydrocortisone was very effective in increasing the worm burdens of chickens infected with Ascaridia galli. No such increase in worm burden was observed when the drug was used with Capillaria (Table 4). Further experimentation would be necessary to show that such results are usual with Capillaria. It should be noted that the egg culture used was prepared from whole worms. Experiment 5, which was conducted at the same time using the same egg culture, also yielded infections of a low order (Table 5). Possible, timing of the use of the immunosuppressor was wrong as it has been shown by Johnson (1962) to be very important in producing large worm burdens of Ascaridia galli.

No upper limit on the degree of worm burdens was observed in the present study. The maximum dosage was 400 eggs, and the maximum burden was 223. Other workers have noted worm burdens as high as 5000 per bird (Levine, 1938).

Although Frazier reported infectivity over 90% in most instances the highest infectivity observed in the present study was 83% in Experiment 6 (Table 6), however, most infections were of a lower order. No explanation is readily apparent for this difference.

The variation in worm burdens between experiments is largely unexplained. Several variables were not explored. The possibility

of damage to the infectivity of the eggs by harsh treatment is suggested as a cause for some variation. Frazier reported that the season of the year did not appear to influence worm burdens, and chickens in the 3-8 week age group gave consistent results.

Workers interested in conducting studies with this worm should expect to concentrate on perfection of techniques for obtaining eggs from feces, as other methods do not provide enough eggs for experimental purposes.

The sharp decrease in worm burden noted with some birds of Experiment I is strongly indicative of the longevity of the worm in the laboratory chicken. Such information should be considered in planning and evaluating experiments extended over 40 days post-exposure.

Summary

Techniques for the culture, infection, and recovery of Capillaria obsignata were evaluated. Egg culture methods investigated were (a) incubation of whole worms, (b) reduction of worms to small pieces and incubation of the residue, and (c) concentration of eggs from the feces of infected birds. Incubation of whole worms yielded the most embryonated eggs but showed the least infectivity. Reduction of worms to fragments resulted in the destruction of large numbers of eggs and produced infections of a low order. Eggs were not successfully cultured from fecal extracts but a culture of this type obtained from another laboratory showed high infectivity. Infection of birds from stock solutions with distilled water or 1.27 M. sucrose solution was

accomplished satisfactorily. A recovery technique using a high pressure spray of water to wash the excised and opened intestine was found to be highly satisfactory. Another technique using a 1% solution of HCl to disrupt the mucosa, followed by settling in a large funnel was found to be unsatisfactory. The use of an immunosuppressant (hydrocortisone) did not increase worm burdens in one experiment. Recommendations are made for other workers interested in the use of C. obsignata as an experimental parasite.

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METHODS FOR THE CULTURE, INFECTION, AND RECOVERY OF *CAPILLARIA OBSIGNATA* MADSEN, 1945

by

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Capillaria obsignata Madsen, 1945, has been known for a number of years as a parasite of chickens, yet there have been few experimental studies using this worm due to the difficulty of laboratory propagation. Methods for egg culture, infection, and recovery were used and evaluated. Egg cultures prepared from worms chopped to small pieces yielded 3.75 embryonated eggs per worm. Cultures prepared from worms ground with the TenBroeck tissue grinder yielded 7.05-14.70 embryonated eggs per worm. Cultures prepared from worms by the use of a VirTis homogenizer yielded 8.86 and 21.36 embryonated eggs per worm. Cultures of whole worms yielded 28.24 embryonated eggs per worm. The preparation of egg cultures by concentration of eggs in the feces of infected chickens was attempted by variations of methods described by other workers and by newly contrived procedures. All methods involved blending feces from infected chickens with water, then removing the coarse material with sieves. Various flotation and sedimentation steps followed. The resulting concentrates did not contain significant numbers of eggs.

The egg cultures were further evaluated by subsequent infection of chickens. The use of sucrose solution for suspension of eggs for inoculation into chickens was investigated. Inoculation techniques were conditioned by the type of egg culture used. Recovery of worms from infected birds could be satisfactorily accomplished with the use of a high pressure spray of water to loosen the worms from the mucosa of the excised intestine.

The results of experimental infections showed that cultures prepared from whole worms were of lower infectivity than cultures

prepared from free eggs. Cultures prepared by chopping, grinding with the TenBroeck tissue grinder or fragmentation with the VirTis homogenizer produced infections of a low order. Worm burdens and infectivity observed were not of the magnitude of those reported by other workers. An immunosuppressant (hydrocortisone), used in one experiment did not increase the worm burden when chickens were infected with eggs in whole worms.